Bumblebees depend on ericaceous species to survive in temperate heathlands

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Abstract. 1. Bumblebees are the predominant wild pollinators for many plant species in temperate regions. A bumblebee colony requires pollen and nectar throughout its lifetime, but degraded and fragmented habitats may have gaps in the temporal and spatial continuity of floral resources. 2. Heathlands are open biotopes that provide favourable habitat for bumblebees like Bombus jonellus, a declining species in Belgium. In heathlands, ericaceous species are the main plants that provide pollen and nectar for bumblebees. Although the nectar composition of ericaceous species has been previously studied, data on pollen composition remain scarce. 3. We examined bumblebee diets (composition of their pollen loads) in Belgian heathlands over the course of a colony lifetime to assess the fidelity of bumblebees for ericaceous species. We compared nutritional values by investigating the chemical composition (amino acids, polypeptides and sterols) of the pollen of the ericaceous and dominant non-ericaceous species present in pollen loads. No relationship was detected between the abundance of a particular plant species in bumblebee loads and its pollen composition. 4. The successive flowering periods and the nutritional quality of pollen of ericaceous species offer valuable resources for bumblebees. Ericaceous species represent a large part of bumblebee diets in heathlands, especially in early spring and late summer when the diversity of other flowering species was low. 5. Bumblebee pollen loads also contained non-ericaceous flowering species that grow outside heathlands. Thus, land planning must incorporate conservation strategies for the different elements of the landscape matrix, including heathlands, peatlands, meadows and margins.

Key words. Bombus jonellus, bumblebee diet, nectar, pollen.

Introduction

Pollinators play an important role in most terrestrial ecosystems, contributing to the reproduction of about 78% of plant species in temperate regions (Ollerton et al., 2011). Among pollinators, bumblebees (Hymenoptera, Apidae, Bombus) are the predominant wild bee pollinators for many wildflowers and crops (Corbet et al., 1991; Osborne & Williams, 1996; Baldock et al., 2015). Their conservation has therefore received major attention in recent years (Goulson et al., 2015), even as alarming evidence about their worldwide decline has accumulated (Rasmont et al., 1993; Kosior et al., 2007; Grixti et al., 2009; Cameron et al., 2011; Carvalheiro et al., 2013; Nieto et al., 2015). This population decline involves multiple causes, but one primary factor concerns modifications...
of the landscape matrix (Hendrickx et al., 2007; Goulson et al., 2010; Bennett & Isaacs, 2014), including destruction, fragmentation and degradation of habitats (Rathcke & Jules, 1993; Fahrig, 2003; Harris & Johnson, 2004). These alterations of habitats through modifications of landscape use (urbanisation, agricultural practices) cause shifts or decreases in the quantity and/or quality of floral resources (i.e. pollen and nectar) available for bumblebees (Biesmeijer et al., 2006; Kleijn & Raemakers, 2008; Goulson et al., 2015).

Bumblebees are entirely dependent on floral resources for survival. Nectar consists of a blend of sugars and represents the major energy source for adult bumblebees; pollen consists mostly of proteins and lipids (Roulston & Cane, 2000) and represents the major nutrient source for larval development (Génissel et al., 2002; Kitaoka & Nieh, 2008). Among lipids, insects use phytosterols in several key metabolic pathways such as the synthesis of molting (Behmer & Nes, 2003) and ovariole maturation (Bloch- enissel et al., 2007; Behmer & Nes, 2003; Alaux et al., 2010). Protein content alone, however, is not sufficient to define the nutritional value of pollen for bees, as it also depends on the amino acid composition (Cook et al., 2003; Nicolson, 2011; Moerman et al., 2015). As bumblebees cannot synthesize essential amino acids and phytosterols de novo, they rely exclusively on pollen for these essential compounds (de Groot, 1953; Behmer & Nes, 2003).

Bumblebees do not store much food in their nests, therefore, they require continuous access to floral resources throughout the lifetime of the colony, from early spring to late summer (Goulson et al., 2005). In spring, in the nest-founding phase, the newly emerged queens eat both nectar and pollen (Prŷs-Jones & Corbet, 1987), using the nutrients in pollen to complete ovary development and start egg production (Génissel et al., 2002; Human et al., 2007). In the colony development phase, after the workers begin to eclose, the queen continues to lay eggs while the workers directly affects reproduction, growth, immunocompetence and longevity of insects (Gilbert, 1981; Smeets & Duchateau, 2003; Alaux et al., 2010). Protein content alone, however, is not sufficient to define the nutritional value of pollen for bees, as it also depends on the amino acid composition (Cook et al., 2003; Nicolson, 2011; Moerman et al., 2015). As bumblebees cannot synthesize essential amino acids and phytosterols de novo, they rely exclusively on pollen for these essential compounds (de Groot, 1953; Behmer & Nes, 2003).

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Disruption in the continuity of flowering resources can threaten bumblebee life cycles, for example, in the fragmented habitats currently typical of heathlands. Heathlands are open, semi-natural habitats dominated by ericaceous species (the heather family) and were widespread in Europe during the 18th and 19th centuries due to human activities like grazing of livestock (Gimingham, 1960). In the late 20th century only a small part of the European heathlands remains (Rebane et al., 1997); most of these biotopes have been converted to agricultural uses or forested areas like spruce plantations (Aerts & Heil, 1993; Webb, 1998). The remaining heathlands constitute a refuge for many rare species and are subject to European legislation to protect their biodiversity (Birds Directive 79/409/EEC – European Commission, 1979 and Habitats Directive 92/43/EEC – European Commission, 1992).

Heathlands support populations of several specialist pollinators and provide major habitat for Bombus jonellus Kirby, a declining bumblebee species especially threatened in Belgium (Rasmont et al., 1993). The destruction of heathlands, as well as their low floral diversity (Forup et al., 2007), suggest that the availability of floral resources for bumblebees may have temporal or spatial gaps that could threaten bumblebee survival.

Species belonging to the Ericaceae are the main entomophilous plant family in the heathlands. Most ericaceous species have poricidal anthers (Hermann & Palser, 2000; Jacquemart, 2003), which release pollen only in response to vibration, termed ‘buzz pollination’ (Buchmann, 1983). Buzz pollination is energetically costly but potentially advantageous in terms of nutritional quality or reduced competition, as only a few insects, such as bumblebees, can pollinate these plants. In recent years, many studies have examined how pollen composition affects the attraction of pollinators (Roulston & Cane, 2000; Aupinel et al., 2001; Cook et al., 2003; Human et al., 2007; Vanderplanck et al., 2014b), but most studies have focused on species with easily accessible pollen, rather than buzz-pollinated species (Roulston et al., 2000; Vanderplanck et al., 2014a). Roulston et al. (2000) observed that buzz-pollinated taxa contain pollen particularly rich in proteins. Other studies, however, showed differences in pollen composition among ericaceous species (Vanderplanck et al., 2014b; Moquet et al., 2015).

In this study, we used pollen loads to analyse the diet of bumblebee individuals visiting flowers of ericaceous species throughout the lifetime of the bumblebee colony. By assessing the quantity and quality of pollen and nectar produced by the species present in the bees’ diet, we determined whether these plants provide valuable resources for bumblebee colonies. We addressed three questions: (i) Do bumblebees use the floral resources of all ericaceous species throughout the colony lifetime? We hypothesised that ericaceous species form a major part of the diet of bumblebees in heathlands, (ii) Do the pollen and nectar of ericaceous plants constitute valuable resources? Bumblebees discriminate between resources according to their quality; therefore, we expected ericaceous species to provide nectar and pollen of high nutritional quality, and (iii) Is the nutritional content of ericaceous pollen linked to bumblebee fidelity, i.e., the constancy of an individual to a particular flowering species? We hypothesised that low-quality pollen would induce bees to collect pollen from other species.
Methods

Bumblebee observations

Studied sites and periods of observations. Observations and sampling were carried out in 10 wet heathland sites located in the Upper Ardenne, Belgium (Table 1). These sites contained 7 of the 8 Belgian Ericaceae: Andromeda polifolia L., Calluna vulgaris L. Hull, Erica tetralix L., Vaccinium myrtillus L., V. oxyccoccos L., V. uliginosum L. and V. vitis-idaea L. We observed the bumblebee visitors to the four most abundant ericaceous species in heathlands, namely, in the order of flowering, Vaccinium myrtillus, V. vitis-idaea, Erica tetralix and Calluna vulgaris (named hereafter ‘target plant species’), for three successive years (2013–2015). Observations were performed on V. myrtillus between 14 May 2013 and 5 June 2013 and between 9 April 2014 and 16 May 2014. Observations on V. vitis-idaea, the second main flowering species, were performed between 14 June 2013 and 2 June 2013, between 19 May 2014 and 11 June 2014 and between 2 June 2015 and 25 June 2015. Observations on E. tetralix were performed between 9 July 2013 and 19 July 2013, between 24 June 2014 and 6 August 2014 and between 25 June 2015 and 20 August 2015. Observations on the last flowering species, C. vulgaris, were performed between 9 August 2013 and 21 August 2013, between 5 August 2014 and 24 August 2014 and between 24 July 2015 and 26 August 2015.

Observations were separated along the season based on bumblebee colony phases. We defined three phases of bumblebee colony lifetime and delimited them depending on the dominant caste present during observations. In this way, we delimited (i) the nest-founding phase, which corresponded to observations on V. myrtillus when bumblebee queens were abundant; (ii) the colony development phase, which corresponded to observations on V. vitis-idaea and E. tetralix when workers were dominant; and finally, (iii) the mating phase, which corresponded to observations on C. vulgaris when newly emerged queens and males were dominant.

Bumblebee diversity. For each of the four successive target plant species, observations took place on sunny, windless days in 6 or more of the 10 sites, depending on the availability of the target plant species. Bumblebee visitors were recorded during one to four entire days per site, per year, and for each target plant species (Table 1). Surveys were carried out on 10 m² plots of continuous shrub cover representative of the population for 20 minutes each hour between 9:00 a.m. and 6:00 p.m. (Mayer et al., 2012). A total of 125 days or 354 h of observations were conducted over the 3 years. A total of 2980 bumblebees were collected with an insect net and were identified. We noted sex and caste of individuals, and whether they carried corbicular pollen loads. We released them on the plot immediately after the 20-min period of observations. Due to their high morphological similarity, individuals of B. terrestris, B. lucorum, B. cryptarum and B. magnus were pooled into one operational taxonomic unit (B. terrestris OTU) and B. hortorum and B. jonellus into another OTU (B. hortorum OTU, Terzo & Rasmont, 2007). At the end of the observation periods, several individuals of each Bombus morphotype were killed (102 individuals in total) with ethyl acetate for identification in laboratory based on an identification key (Rasmont & Terzo, 2010).

Pollen loads. In 2013 and 2014, during the 20-min periods of observations, bumblebees with pollen loads visiting target plant species were immobilised in a beemarking cage and one of the two pollen loads was carefully removed using a toothpick. In the laboratory, pollen loads were weighed to check the influence of load weight on taxa diversity. The samples were then acetolysed (Erdman, 1960, modified) for palynological analyses by light microscopy (Leitz Wetzlar). Pollen grain identification was based on a reference collection from the Université catholique de Louvain, an identification key (Reille, 1992) and a comprehensive list of flowering plants in the studied sites. A total of 343 pollen loads was analysed. To detect all the pollen species present and to accurately estimate the proportions of the different pollen species, a minimum of 400 randomly chosen pollen grains were identified per vortexed pollen load sample, as in other recent studies (Mayer et al., 2012; Moisan-Deserres et al., 2014).

Floral diversity. To facilitate and increase accuracy of pollen identification in bumblebee loads, we recorded all other flowering entomophilous plant species that cover more than 1 m². Floral diversity surveys were carried out just before bumblebee observations within a radius of 200 m around the studied plots.

Assessment of pollen and nectar quality

Floral pollen collection. At the peak of flowering, that is, between early April and late August, stems (30–200 depending on plant species, number of flowers per stem and the quantity of pollen per flower) of the four target plant species and of the main non-ericaceous species detected in bumblebee pollen loads were harvested from a minimum of five individual plants per site in six sites. Only ericaceous species with population size and density allowing enough pollen collection were studied. Stems were kept in tap water for one night at room temperature (approximately 20 °C) in the laboratory. The next morning, stamens were extracted from newly open flowers and dried at room temperature for 12 h after which pollen was removed using a sieve (Sieve 3”, Brass–Stainless, Full Height, 80 μm). Because the majority of the ericaceous species studied (V. myrtillus, V. uliginosum and E. tetralix) had poricidal anthers, pollen was collected from the flowers by vibration. A small vibrator was built out of a 5-mm rod, which was slightly inclined and attached to a small handle (Sami Yunus; Institute of Condensed Matter

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Table 1. Location and size of studied sites situated in the Upper Ardenne, Belgium.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Grande Fange</th>
<th>Wé des Pourceaux</th>
<th>Pisserotte Crépale</th>
<th>Fanges aux Mochettes</th>
<th>Grand Passage Pouhon</th>
<th>Robiéfa Nazieuf</th>
<th>Sacrawe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coordinates</td>
<td>50°14'40&quot;N 5°46'45&quot;E</td>
<td>50°14'42&quot;N 5°44'57&quot;</td>
<td>50°13'13&quot;N 5°47'54&quot;E</td>
<td>50°16'40&quot;N 5°44'07&quot;E</td>
<td>50°13'21&quot;N 5°40'55&quot;E</td>
<td>50°13'44&quot;N 5°45'46&quot;E</td>
<td>50°14'32&quot;N 5°41'51&quot;E</td>
</tr>
<tr>
<td>Localities</td>
<td>Vielsalm</td>
<td>Vielsalm</td>
<td>Gouvy</td>
<td>Lierneux</td>
<td>La Roche-en-Ardenne</td>
<td>Houffalize</td>
<td>Manhay</td>
</tr>
<tr>
<td>Natural reserve area (ha)</td>
<td>282</td>
<td>NA</td>
<td>206</td>
<td>30</td>
<td>71</td>
<td>165</td>
<td>57</td>
</tr>
<tr>
<td>Heathland area (ha)</td>
<td>0.31</td>
<td>1.15</td>
<td>1.6</td>
<td>7.63</td>
<td>9.58</td>
<td>10.91</td>
<td>18.55</td>
</tr>
<tr>
<td>Days of observation of V. myrtillus</td>
<td>2013</td>
<td>1</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>2</td>
<td>–</td>
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<td></td>
<td>2014</td>
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<td>–</td>
<td>1</td>
<td>–</td>
<td>3</td>
<td>–</td>
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<tr>
<td>Days of observation of V. vitis-idaea</td>
<td>2013</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>2</td>
<td>–</td>
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<td></td>
<td>2014</td>
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<td>2</td>
<td>–</td>
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<tr>
<td>Days of observation of E. tetralix</td>
<td>2013</td>
<td>2</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>2</td>
<td>–</td>
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<td>2015</td>
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<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Days of observation of C. vulgaris</td>
<td>2013</td>
<td>2</td>
<td>–</td>
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<td>1</td>
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The years of observation of bumblebee visits are detailed for each of four target plant species with number of observation days. Sites are arranged by increasing area of heathland.
and Nanosciences, Université catholique de Louvain). Rod rotations could be modulated to produce vibrations between 200 and 800 Hz. Pure pollen samples were pooled by species to reach at least 200 mg (the amount required for chemical analyses), lyophilised and stored at –20 °C.

Chemical composition of pollen. The polypeptide content (molecular weight >10 000 Da) was quantified from 5 mg dry pollen in triplicate for each species following the method described by Vanderplanck et al. (2014a). The quantification of total polypeptide content was performed using the standard curve of the BCA Protein Assay Kit (Pierce, Thermo Scientific), at the University of Mons (Mons, Belgium).

The amino acid content was quantified from 3 mg dry pollen in triplicate for each species following the method described in Vanderplanck et al. (2014a). Total amino acids were measured separately by ion exchange chromatography and post-column ninhydrin derivatisation (Biochrom 20 plus amino acid analyser) at the University of Liège (Gembloux, Belgium). Only tryptophan was omitted because its isolation requires separate alkaline hydrolysis from an additional amount of sample. Essential amino acids for bumblebees were assumed to be the same as those identified for honeybees (i.e. arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine; de Groot, 1953).

Phytosterol content was quantified from 15 mg of dry pollen for each species following the method described in Vanderplanck et al. (2011). The total phytosterol content was determined considering all quantifiable peaks of sterols that eluted between cholesterol and betulin (internal standard) at the University of Liège (Gembloux, Belgium). Identities were made by comparing the relative retention times with those of a sunflower oil reference. Due to technical problems, we were unable to provide total sterol content for Salix x multirnervis.

Pollen volume per flower. Because pollen grains vary widely in size, pollen volume provides a better indicator of the nutrient storage capacity than the number of pollen grains (Buchmann & O’rourke, 1991). For the main plant species present in the bumblebee pollen loads, we collected flower buds from 10 individuals in two different populations. Buds were fixed in FAA (ethanol 70% : formaldehyde 35% : acetic acid, 8:1:1) before counting. To extract pollen, anthers were dissected, dropped into a known volume of Alexander’s red solution (300–3200 μl, depending on pollen quantity), crushed with a plastic rod, sonicated and vortexed. A 5-μl drop of pollen suspension was deposited on slides and all pollen grains were counted. Pollen dimensions were measured with Motic Images Plus (Version 2.0) software from pictures taken with a Moticam2000 camera using light microscopy (Nikon Eclipse E400) at a magnification of 450×. Pollen volumes (V) were calculated using the measured longitudinal (l) and equatorial (e) lengths according to the formula $V = \frac{1}{6}lre^3$ for spheroidal grains and $V = \frac{1}{6}lre^2$ for ellipsoidal grains (Buchmann & O’rourke, 1991; Rasheed & Harder, 1997).

Nectar volume and composition. We sampled nectar at the peak of flowering for each target plant species. In 2014, on at least 6 days per plant species with optimal weather conditions (no rain in 24 h), glass capillary tubes of 0.5 μl or 1 μl (Hirschmann Laborgeräte, Eberstadt, Germany) were used to collect nectar from a minimum of 10 freshly opened flowers from five bushes. Depending on the species, between 60 (Vaccinium spp.) and 720 flowers (C. vulgaris) were sampled. The nectar volume was estimated by measuring the length of the nectar column in the capillary tube. In the laboratory, nectar tubes were pooled by species and site to reach at least 0.5 mg and stored at –80 °C before analyses. Sugar composition was determined by gas chromatography with a PerkinElmer Autosystem XL equipped with a split injector (1/20) and helium as carrier gas (flow of 1 ml/min). The injector and detector temperatures were maintained at 250 and 350 °C respectively. Sugar (i.e. sucrose, glucose and fructose) analyses for nectar composition were performed at the Centre Apicole de Recherche et d’Information (CARI, Louvain-la-Neuve, Belgium).

Statistical analyses

All analyses were conducted in R (R Development Core Team, 2013) and, if not indicated otherwise, data are presented as mean ± standard deviation.

For each target plant and bumblebee species, we excluded loads from the statistical analyses when n < 5. We also excluded cuckoo bumblebees (subgenus Psithyrus) due to their different foraging behaviour (i.e. no worker caste and no pollen collection, Prýs-Jones & Corbet, 1987). To determine whether the proportion of bumblebee individuals collecting pollen, proportion of pollen derived from visited plant species and the number of plant species in pollen loads differed per bumblebee species and per plant species visited, we performed generalised linear mixed models (GLMM) analysis with year of observations and studied sites as random factors and with year nested within studied sites. If random effects were not significant, we preferentially conducted GLM analysis. We used a binomial error distribution for proportion of bumblebee individuals collecting pollen, quasi-binomial for proportion of pollen of visited plant species in bumblebee loads and quasi-Poisson for number of taxa in bumblebee loads. To verify the absence of correlation between the plant taxa number in the pollen loads, the weight of pollen loads and the number of flowering plant species around the studied plots, we performed Spearman’s rank correlation tests.

Because assumptions for parametric tests (i.e. normality and homoscedasticity) were not respected, we used Kruskal–Wallis tests to compare polypeptide, amino acid, essential amino acid and phytosterol contents among...
plant species. If an overall significant difference was detected, pairwise comparisons using Tukey and Kramer (Nemenyi) tests were conducted. To test differences in amino acid and sterol composition among pollen types, we performed a permutational multivariate analysis of variance (perMANOVA) using the Euclidean distance matrix and 999 permutations (‘adonis’ command, R-package vegan). Prior to this perMANOVA, the multivariate homogeneity of within-group covariance matrices was verified using the ‘betadisper’ function. Both similarities and dissimilarities in chemical compositions among the different pollen sources were visually assessed using principal component analysis (PCA). In PCA calculated on the percentage of total amino acids in pollen, we added the ideal balance determined by de Groot (1953) for honeybees excluding tryptophan (arginine 11.5%, histidine 5.2%, isoleucine 14.6%, leucine 16.7%, lysine 11.5%, methionine 5.2%, phenylalanine 9.4%, threonine 11.5% and valine 14.6%), as an illustrative individual.

Results

Fidelity for ericaceous floral resources throughout the colony lifetime

Foraging behaviour on ericaceous plant species. Bumblebees were observed on the ericaceous plants species throughout the colony lifetime from April on V. myrtillus to August on C. vulgaris. Beside the subgenus Psithyrus, six different operational taxonomic units (OTUs) were observed using ericaceous resources: Bombus hortorum OTU (in which 22/27 individuals were identified as B. jonellus Kirby and the remaining 5 as B. hortorum L.), B. lapidarius L., B. terrestris L., B. hypnorum L., B. pascuorum Scopoli and B. pratorum L. Bumblebee species differed significantly in their foraging behaviour (GLMM; $\chi^2 = 51.6; P < 0.001$; Fig. 1). Although B. jonellus collected pollen on all ericaceous species throughout the colony lifetime (except on C. vulgaris), B. pascuorum visited ericaceous species mainly for nectar and collected significantly less pollen than other species (GLMM; $P < 0.05$). Bombus hypnorum individuals displayed intermediate foraging behaviour, as they foraged V. vitis-idaea pollen in heathlands, but did not forage on other ericaceous species.

The proportion of bumblebees collecting pollen differed significantly among the target plant species (GLMM; $\chi^2 = 186.5; P < 0.001$; Fig. 1) from 63% on V. vitis-idaea during colony development (mainly for pollen) to 13% on C. vulgaris at the end of colony lifetime (mainly for nectar). On average, 22% of bumblebees caught on V. myrtillus and E. tetralix carried pollen loads.

Pollen collection. The analysis of both the proportion of the target ericaceous species and the number of other plant species in bumblebee pollen loads revealed that bumblebees collecting pollen showed a high fidelity for ericaceous resources. The proportion of pollen of target plant species (Fig. 2a) was significantly lower for bumblebees caught on V. myrtillus (0.56 ± 0.45; GLMM; $t_{309} = -3.7; P < 0.001$), V. vitis-idaea (0.79 ± 0.33; GLMM; $t_{309} = -3.2; P = 0.02$) and E. tetralix (0.65 ± 0.40; GLMM; $t_{308} = -3.8; P < 0.001$) than for bumblebees caught on C. vulgaris in late summer (0.86 ± 0.26). Among bumblebee species, B. jonellus individuals had a higher proportion of pollen from ericaceous species in their loads (0.80 ± 0.32) compared with B. terrestris OTU (0.70 ± 0.38; GLMM; $t_{308} = -3.15; P = 0.002$) and B. pascuorum (0.55 ± 0.43; GLMM; $t_{309} = -2.97; P = 0.003$).

We did not find any correlation between the mean number of plant taxa in the pollen loads and the weight of pollen loads ($r^2 = -0.10; t_{34} = -0.93; P = 0.35$) or the number of flowering plant species around the studied sites ($r^2 = -0.05; t_{32} = 0.46; P = 0.65$). The number of different plant taxa (Fig. 2b) was higher in loads of bumblebees caught during colony development on V. vitis-idaea (GLM; $t_{308} = 2.5; P = 0.01$) and E. tetralix (GLM; $t_{313} = 2.6; P = 0.009$) than in loads of bumblebees caught during mating phase on C. vulgaris. Among bumblebees, B. jonellus individuals had significantly lower plant taxa diversity in their pollen loads than B. terrestris OTU individuals (GLM; $t_{308} = 2.4; P = 0.03$).

We observed that 63% of pollen loads were monospecific (i.e. >95% conspecific pollen grains) and 83% of them contained only pollen of the target ericaceous species. In addition to the target plant species, bumblebees collected pollen on trees and shrubs like Salix spp. and Sambucus racemosa during the nest-founding phase, on Cytisus scoparius, Narthecium

Fig. 1. Proportions of bumblebees collecting pollen on the four target plant species in the 10 studied sites. Data are shown as mean ± SE. Bombus terrestris OTU (operational taxonomic units) refers to B. terrestris, B. lucorum, B. cryptarum and B. magnus.
ossifragum and Rubus spp. during colony development and on Filipendula ulmaria in the last phase of colony life (Fig. 3). Plant species in the diet of B. jonellus originated mainly from heathlands, peatlands (e.g. Narthecium ossifragum, Salix spp.) and heathland edges (e.g. Cytisus scoparius, Rubus spp., Sambucus racemosa). By contrast, the other bumblebee species foraged pollen on plant species growing in nearby biotopes like meadows and woods (e.g. Malus spp., Vicia spp., Acer spp. or Hypericum spp.; Fig. 3). The presence of plant species in pollen loads coincided with the peak of flowering of each plant (Table 2).

<table>
<thead>
<tr>
<th>Bumblebee colony phases</th>
<th>Nest-founding phase</th>
<th>Colony development</th>
<th>Mating phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target plant species</td>
<td>V. myrtillus</td>
<td>V. vitis-idaea</td>
<td>E. tetralix</td>
</tr>
<tr>
<td>Plant species</td>
<td></td>
<td></td>
<td>C. vulgaris</td>
</tr>
<tr>
<td></td>
<td>Vaccinium spp. (E)</td>
<td>Vaccinium spp. (E)</td>
<td>Vaccinium spp. (E)</td>
</tr>
<tr>
<td></td>
<td>Sambucus racemosa (A)</td>
<td>Malus spp. (R)</td>
<td>Malus spp. (R)</td>
</tr>
<tr>
<td></td>
<td>Cytisus scoparius (F)</td>
<td>Vicia spp. (F)</td>
<td>Cytisus scoparius (F)</td>
</tr>
<tr>
<td></td>
<td>Cotoneaster spp. (R)</td>
<td></td>
<td>Cotoneaster spp. (R)</td>
</tr>
<tr>
<td>B. jonellus</td>
<td>78.0 8.6 12.9</td>
<td>89.8 3.2</td>
<td>5.2</td>
</tr>
<tr>
<td>B. lapidarius</td>
<td>55.9 14.1 29.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. terrestris OTU</td>
<td>44.8 25.9 22.9</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>B. hypnorum</td>
<td>46.0 27.5 11.5</td>
<td>9.2 4.1</td>
<td>52.0 47.0</td>
</tr>
<tr>
<td>B. pratorum</td>
<td>57.3 22.7</td>
<td>19.2 12.2</td>
<td>72.6 12.7 1.2</td>
</tr>
<tr>
<td>Total</td>
<td>56.1 20.9 10.0 5.0</td>
<td>2.8 2.8 1.2</td>
<td>72.0 13.4 3.2</td>
</tr>
</tbody>
</table>

Fig. 2. Fidelity of the bumblebees estimated by pollen load analysis. (a) Proportions of pollen of the target ericaceous plant species and (b) number of the different plant taxa in pollen loads, for each bumblebee species caught on target plant species in the 10 studied sites. Data are shown as mean ± SE. Bombus terrestris OTU (operational taxonomic units) refers to B. terrestris, B. lucorum, B. cryptarum and B. magnus.

Fig. 3. Percentage of pollen grains of the different plant species present in diet of bumblebee individuals caught on Vaccinium myrtillus, V. vitis-idaea, Erica tetralix and Calluna vulgaris in the 10 studied sites. Only pollen of plant species with total percentage > 1 and Bombus species with sample > 5 pollen loads are shown. Plant species indicated in brackets. A: Adoxaceae; E: Ericaceae; F: Fabaceae; H: Hypericaceae; M: Malvaceae, N: Nartheciaceae; R: Rocaceae; Sal: Salicaceae; Sap: Sapindaceae.
Table 2. Flowering periods of the different plant species (adapted from Lambinon & Verloove, 2012) and number and volume (mm³) of pollen grains per flower (n = 10).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Flowering periods</th>
<th>Bumblebee colony phases</th>
<th>Pollen grains/flower</th>
<th>Volume/flower (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salix x multinervis</em></td>
<td>March–May</td>
<td>Nest-founding phase</td>
<td>13921 ± 4830</td>
<td>2.46 ± 0.41</td>
</tr>
<tr>
<td><em>Malus pumila</em></td>
<td>April–May</td>
<td>Nest-founding phase</td>
<td>7125 ± 1258</td>
<td>0.24 ± 0.04</td>
</tr>
<tr>
<td><em>Vaccinium myrtillus</em></td>
<td>April–June</td>
<td>Nest-founding phase</td>
<td>51668 ± 2902*</td>
<td>0.69 ± 0.16</td>
</tr>
<tr>
<td><em>Cytisus scoparius</em></td>
<td>May–July</td>
<td>Colony development</td>
<td>24781 ± 19353</td>
<td>0.36 ± 0.28</td>
</tr>
<tr>
<td><em>Sorbus aucuparia</em></td>
<td>May–July</td>
<td>Colony development</td>
<td>67273 ± 11673</td>
<td>0.7 ± 0.12</td>
</tr>
<tr>
<td><em>Vaccinium uliginosum</em></td>
<td>May–July</td>
<td>Colony development</td>
<td>17760 ± 693*</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td><em>Vaccinium vitis-idaea</em></td>
<td>May–June</td>
<td>Colony development</td>
<td>46794 ± 1818*</td>
<td>0.56 ± 0.09</td>
</tr>
<tr>
<td><em>Rubus</em> spp.</td>
<td>June–July</td>
<td>Colony development</td>
<td>117593 ± 16534</td>
<td>0.96 ± 0.14</td>
</tr>
<tr>
<td><em>Erica tetralix</em></td>
<td>June–August</td>
<td>Colony development</td>
<td>12333 ± 670*</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td><em>Narthecium ossifragum</em></td>
<td>July–August</td>
<td>Colony development</td>
<td>87500 ± 24588</td>
<td>0.26 ± 0.07</td>
</tr>
<tr>
<td><em>Hypericum perforatum</em></td>
<td>July–September</td>
<td>Colony development</td>
<td>684467 ± 293457</td>
<td>1.16 ± 0.5</td>
</tr>
<tr>
<td><em>Filipendula ulmaria</em></td>
<td>July–September</td>
<td>Mating phase</td>
<td>143713 ± 31605</td>
<td>0.8 ± 0.18</td>
</tr>
<tr>
<td><em>Calluna vulgaris</em></td>
<td>July–September</td>
<td>Mating phase</td>
<td>15126 ± 1822*</td>
<td>0.14 ± 0.07</td>
</tr>
</tbody>
</table>

Plant species are ranked in the order of the beginning of the flowering period. Bumblebee colony phase is the period during which pollen grains of the plant species were present in bumblebee loads.

*From Jacquemart (2003).

Pollen and nectar characteristics.

Pollen quantity and composition. The most abundant plant species found in bumblebee pollen loads throughout the colony lifetime were *Acer pseudoplatanus L.*, *Calluna vulgaris* (L.) Hull, *Cytisus scoparius* L., *Erica tetralix* L., *Filipendula ulmaria* L., *Hypericum perforatum* L., *Malus pumila* Mill., *Narthecium ossifragum* L., *Rubus* spp., *Salix x multinvitis Döl*, *Sorbus aucuparia* L., *Tilia cordata* Mill., *Vaccinium myrtillus* L. and *Vaccinium uliginosum* L. Pollen volume per flower (Table 2) varied among species, from 0.09 ± 0.02 mm³ (*Erica tetralix*) to 2.26 ± 0.41 mm³ (*Salix x multinvitis*).

For pollen composition, polypeptide contents differed significantly among species (Kruskal–Wallis; $\chi^2 = 39.7; P < 0.001$; Fig. 4a; Appendix S1). For example, *V. myrtillus* pollen had significantly lower polypeptide contents (18.9 ± 7.0 mg/g) compared to *E. tetralix* (216.5 ± 6.9 mg/g; $P = 0.02$) and *F. ulmaria* pollen (168.7 ± 27.6 mg/g; $P = 0.05$). Total amino acid content also significantly differed among plant species (Kruskal–Wallis; $\chi^2 = 33.0; P = 0.002$; Fig. 4b). For example, *H. perforatum* pollen had higher amino acid content (434.9 ± 16.3 mg/g) than *M. pumila* (215.5 ± 14.5 mg/g; $P = 0.001$) and *T. cordata* (243.1 ± 13.2 mg/g; $P = 0.02$). Likewise, the essential amino acid composition of pollen differed significantly among plant species (PerMANOVA; $F_{13,29} = 12.5; P = 0.001$; Appendix S2). This difference was visually assessed by PCA (Fig. 5), where plant species clustered into three groups: (i) *H. perforatum* had a high proportion of threonine and methionine and a low proportion of isoleucine and leucine; (ii) *F. ulmaria*, *Rubus* spp. and *V. uliginosum*, had a high proportion of lysine and a low proportion of phenylalanine; (iii) the last cluster included species with essential amino acid composition close to the ideal balance established by de Groot (1953).

Like protein content, sterol content (Kruskal–Wallis; $\chi^2 = 25.4; P = 0.01$; Fig. 4c) and composition (PerMANOVA; $F_{13,29} = 4.4; P = 0.001$; Appendix S3) of pollen significantly differed among plant species. As illustrated by PCA (Fig. 6), plant species clustered into three groups. The first axis separated *Salix x multinvitis* (i) from the other species and the second axis separated plant species in two groups, one (ii) composed of *A. pseudoplatanus*, *C. scoparius*, *C. vulgaris*, *E. tetralix*, *H. perforatum*, *N. ossifragum*, *T. cordata* and *V. myrtillus*, had high proportion of $\beta$-sitosterol (30–65%) and/or $\delta$ 5-avenasterol (17 to 47%). In this group, pollen of some species also had a high percentage of $\delta$-avenasterol (*E. tetralix* 27%, *N. ossifragum* 14%, *V. myrtillus* 12% and *C. vulgaris* 9%). The other group (iii) included the remaining species and had pollen with a high proportion of 24-methylenecolesterol and campesterol.

Nectar quantity and composition. The nectar of the different ericaceous species had similar sugar concentrations (Kruskal–Wallis; $\chi^2 = 6.9578$; d.f. = 4; $P = 0.14$; Table 3), but differed in quantity per flower (Kruskal–Wallis; $\chi^2 = 76.0; P < 0.001$). *Vaccinium myrtillus* produced more nectar per flower (0.77 ± 1.38 µl) than *V. vitis-idaea* (0.12 ± 0.26 µl), *E. tetralix* (0.20 ± 0.29 µl) and *C. vulgaris* (0.01 ± 0.01 µl); indeed, *C. vulgaris* produced significantly less nectar than any other target ericaceous species. *Vaccinium myrtillus* nectar predominantly contained sucrose (87.7 ± 5.2%), whereas *V. uliginosum*, *V. vitis-idaea* and *C. vulgaris* nectar predominantly contained hexoses (fructose + fructose). *Erica tetralix* had nectar with high proportions of both fructose and sucrose.

Despite differences in pollen quantity and quality, we did not detect any correlation between the proportions of pollen in bumblebee loads and the composition of pollen (polypeptide, amino acid and sterol contents) or quantity of pollen (pollen volume per flower; \( P > 0.05 \)). Fidelity to target plant species (proportion of visited target plant species and number of taxa in pollen loads) and proportion of bumblebees collecting pollen were not linked to nutrient concentrations. Bumblebee loads had similar proportion of pollen of *V. myrtillus* (0.56 ± 0.45) and *E. tetralix* (0.65 ± 0.40), despite the large difference of polypeptide concentration in their pollen (18.9 ± 7.0 vs. 216.6 ± 6.9 mg/g respectively).

In the same way, the collection of nectar was not linked to the quantity of nectar in the flower and to sugar concentration. *Calluna vulgaris*, a species visited mainly for nectar, produced the lowest quantity of nectar and this nectar had intermediate sugar concentration.

**Discussion**

*Do ericaceous species offer sufficient pollen resources for bumblebees throughout the entire colony lifetime?*

Bumblebees have been reported as the main visitors to all the target ericaceous species except *C. vulgaris*, a more generalist plant (Ritchie, 1955, 1956; Jacquemart, 1993; Mahy et al., 1998; Mayer et al., 2012). In this study, bumblebees visited ericaceous species for pollen and/or nectar, but not all ericaceous species were visited for the same floral resources: the bumblebees mainly visited *V. vitis-idaea* for pollen and *C. vulgaris* for nectar. The different flowering periods could explain these differences, since the flowering period of *V. vitis-idaea* corresponds to bumblebee colony development (Prýs-Jones & Corbet, 1987), when pollen collection is important for larval growth (Ribeiro et al., 1998; Pelletier & McNeil, 2003). By contrast, the flowering period of *C. vulgaris* corresponds to the mating phase and the end of the colony lifetime, when males and queens forage for their own needs (Prýs-Jones & Corbet, 1987). Furthermore, bumblebee fidelity for pollen from the studied heathland species was higher in the early spring on *S. x multijervis* (Moquet et al., 2015) and in the late summer on *C. vulgaris*. These results suggested that the food web of bumblebees in heathlands is highly limited (Mayer et al., 2012) during these two periods.

The foraging behaviour differed according to bumblebee species. Ericaceous species were the main sources of...
both pollen and nectar for *B. jonellus* throughout the colony lifetime and around 40% of individuals carried pollen loads with a mean of 80% of ericaceous pollen grains. *Bombus jonellus* showed higher fidelity for ericaceous species than did other bumblebee species, particularly *B. terrestris*. Moreover, the proportion of heathland plant
species was higher in the diet of B. jonellus than in the diet of other bumblebee species. These results can be explained by B. jonellus having a more restricted preference for foraging on ericaceous species (Goulson et al., 2005; Kleijn & Raemakers, 2008) or having smaller foraging distances compared with B. terrestris (Martin et al., 2005; Westphal et al., 2006).

By contrast, other bumblebee species showed lower fidelity to ericaceous species. For example, B. pascuorum visited ericaceous species mainly for nectar, as a low proportion of observed individuals had pollen loads. Bombus hypnorum, which usually prefer foraging from tree species (Crowther et al., 2014), was observed in large amounts on V. vitis-idaea, where individuals mainly collected pollen. Like B. pascuorum, B. hypnorum was dependent on the surrounding biotopes for one flower resource or for both pollen and nectar. Moreover, plant species present in the pollen loads indicated that bumblebees move between different elements in the landscape mosaic, including heathlands, peatlands, meadows and their margins. This result highlights the importance of heterogeneity for bumblebee foraging in a mosaic landscape (Westphal et al., 2006; Goulson et al., 2010; Somme et al., 2014; Senapathi et al., 2015).

Is the nutritional composition of pollen valuable throughout the colony lifetime?

The pollen of plant species differed significantly in polypeptide, amino acid and sterol contents. For example, E. tetralix and F. ulmaria pollen were characterised by high polypeptide but pollen of V. myrtillus, which flowered during bumble bee nest foundation, had a very low polypeptide contents. In contrast to the previous study from Roulston et al. (2000), our study showed a significant difference in polypeptide contents among species of the same plant family (Ericaceae) and high variability among species of the same genus (Vaccinium). Despite the differences in polypeptide concentrations, the amino acid and essential amino acid concentrations of the different ericaceous pollens were high and remained similar throughout colony lifetime. We, however, observed differences in amino acid contents among other species found in pollen loads. For example, H. perforatum had a higher amino acid content than M. pumila and T. cordata. The majority of pollen had comparable essential amino acid compositions, although several species present in significant proportion in pollen loads had pollen with lower concentrations of some essential amino acids, for instance H. perforatum, F. ulmaria and Rubus spp.

The sterol content of ericaceous pollen was similar throughout colony lifetime. Sterol contents of the diet can affect insect growth (Pilorget et al., 2010; Vanderplanck et al., 2014b), but the quantity needed for bumblebee growth remains unknown. Our studied plant species can be separated into two groups according to sterol composition: the first group included the target ericaceous species, in which β-sitosterol and/or δ5-avenasterol were dominant and the second group included species in which 24-methylenecholesterol was dominant. High contents of 24-methylenecholesterol, β-sitosterol or δ5-avenasterol can promote the development of bigger larvae in B. terrestris (Vanderplanck et al., 2014b). The essential sterol 24-methylenecholesterol influences moulting and the development of ovaries (Svoboda et al., 1978, 1980; Human et al., 2007), and β-sitosterol and δ5-avenasterol have a phagostimulant effect on bumblebee species (Rasmont et al., 2005). Pollen of E. tetralix, N. ossifragum, V. myrtillus and C. vulgaris contained a significant amount of δ7-avenasterol (9–27%). δ7-Phytosterol might act as a chemical protectant for pollen, as it has been reported to be detrimental to herbivorous insects lacking the enzymes necessary to completely convert δ7-sterols to δ5-sterols (Janson et al., 2009; Sedivy et al., 2011). Therefore, the target ericaceous species studied here provide valuable resources throughout the colony lifetime. In fact, all studied species had pollen with total amino acid contents higher than 20%, a value considered to promote the development of bee larvae (Génissel et al., 2002; Tasei & Aupinel, 2008; Vanderplanck et al., 2014a).

Nevertheless, V. myrtillus pollen had relatively low polypeptide contents, and some species having pollen rich in δ7-sterol, which may be detrimental for development of bee larvae.

Is the nutritional composition of nectar from ericaceous species valuable throughout the colony lifetime?

Bees do not often collect pollen and nectar simultaneously, but typically visit different plant species for each

---

**Table 3.** Nectar production of the five target ericaceous plant species: quantity of nectar per flower (μl; mean ± SD), total sugar content (g/100 g; mean ± SD) and glucose, fructose and sucrose percentage.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Quantity per flower (μl)</th>
<th>Sugar content (g/100 g)</th>
<th>Glucose (%)</th>
<th>Fructose (%)</th>
<th>Sucrose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. myrtillus</td>
<td>0.8 ± 1.4</td>
<td>17.9 ± 6.1</td>
<td>4.7 ± 2.9</td>
<td>7.6 ± 2.5</td>
<td>87.7 ± 5.2</td>
</tr>
<tr>
<td>V. uliginosum</td>
<td>0.3 ± 0.4</td>
<td>27.4 ± 11.2</td>
<td>40.2 ± 2.3</td>
<td>59.0 ± 1.7</td>
<td>0.8 ± 0.6</td>
</tr>
<tr>
<td>V. vitis-idaea</td>
<td>0.1 ± 0.3</td>
<td>21.1 ± 12.8</td>
<td>44.9 ± 1.6</td>
<td>52.6 ± 1.3</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>E. tetralix</td>
<td>0.2 ± 0.3</td>
<td>6.0 ± 3.8</td>
<td>7.9 ± 1.1</td>
<td>44.9 ± 3.4</td>
<td>47.2 ± 2.3</td>
</tr>
<tr>
<td>C. vulgaris</td>
<td>0.0 ± 0.0</td>
<td>16.6 ± 6.7</td>
<td>43.4 ± 1.5</td>
<td>53.3 ± 0.7</td>
<td>3.3 ± 0.8</td>
</tr>
</tbody>
</table>

All sites were pooled. n = 3 replicates for nectar ratio and composition. n > 60 floral units for nectar quantities. Plant species are ranked in the order of the flowering period.
resource (Brian, 1957). In our study, some ericaceous species like *C. vulgaris* were mainly visited for nectar.

The sugar content of nectar did not differ significantly among species and was lower than 40%, the concentration usually preferred by bumblebees (Harder, 1986; Cnaani et al., 2006). Consequently, total sugar content cannot explain differences in preference among the bumblebee species studied. Here, we found that the quantity of nectar differed among plant species, with *V. myrtillus* flowers providing significantly more nectar than other species. It is possible that, because *V. myrtillus* had low pollen quality in comparison to other species, high nectar quantity is the main attractive reward. In contrast, *C. vulgaris* produced very little nectar per flower, but due to its high flower density (up to 7900 flowers per individual, Beijerinck, 1940; Gimmingham, 1960), the total quantity remained attractive.

The sugar composition of nectar also differed among ericaceous species. Nectar of *V. myrtillus* was sucrose dominant, whereas nectars of *V. uliginosum*, *V. vitis-idaea* and *C. vulgaris* were hexose dominant. Different sugar compositions in nectar within the same family or genus have been described previously (Barnes et al., 1995; Krömer et al., 2008). The nectar of *E. tetralix* is quite unusual, even within the *Erica* genus, offering both fructose and sucrose in large amounts (Baker & Baker, 1990; Barnes et al., 1995; Krömer et al., 2008). In contrast to other studies (Percival, 1961; Baker & Baker, 1983, 1990; Krömer et al., 2008), we did not find any relationship between the nectar composition and floral visitors (i.e. bumblebees for all target plants) or between the nectar composition and floral morphology (e.g. *V. vitis-idaea* and *C. vulgaris* have open flowers).

**What governs bumblebee fidelity and choice of pollen resources?**

We observed no correlation between the abundance of pollen in bumblebee loads and the concentrations of polypeptides, amino acids and sterols or the pollen volume. Similarly, neither the proportion of bumblebees collecting pollen of ericaceous species nor the fidelity was affected by polypeptide, amino acid or sterol concentrations. For example, the bumblebees showed similar fidelity for *V. myrtillus* and *E. tetralix*, despite the large difference in polypeptide concentration in their pollen. This observation disagrees with results of other studies showing the importance of pollen quality in bumblebee choices (Robertson et al., 1999; Hanley et al., 2008; Kitaoka & Nieh, 2008; Moquet et al., 2015). It is now clear that bumblebees can distinguish between pollen with low or high protein content (Ruedenauer et al., 2015). Bumblebees can use this capacity to choose plants with high pollen quality when they have a binary choice (Robertson et al., 1999; Kitaoka & Nieh, 2008; Moquet et al., 2015; Quinet et al., 2016).

In field observations, when many flowering species are available, bumblebee choices were less clear (Rasheed & Harder, 1997).

The lack of correlation between pollen quality and the proportion of specific pollen in the bumblebee diet can be explained by the need to mix pollen of different origins. Pollen mixing increases the quality of the overall diet by improving the nutrient balance (Bernays et al., 1994; Eckhardt et al., 2013). In our study, some bumblebee species supplemented pollen poor in 24-methylenecolesterol but rich in δ7-sterols (from *V. myrtillus*, *E. tetralix* and *C. vulgaris*) with pollen rich in 24-methylenecolesterol and campesterol (from *F. ulmaria*, *Malus pumila* or *Rabun* spp.). In the same way, the bumblebees combined different pollen amino acid profiles. Moreover, Rasheed and Harder (1997) showed that pollen selection is not only exclusively dependent on pollen composition, but also depends on other factors such as plant density (Kunin, 1997) or spatial distribution (Jha et al., 2013).

**Conclusion**

Our results demonstrate that heathlands provide valuable habitats for bumblebees due to the succession of flowering periods and the presence of suitable nutrients in pollen and nectar of ericaceous species. The high fidelity of bumblebees, particularly of *B. jonellus*, for ericaceous species showed that these plant species can constitute a substantial part of the bumblebee pollen diet in heathlands. Nevertheless, despite European legislation, the quality and quantity of heathland continues to decrease in Europe (Romão et al., 2015). Estimation of the minimal heathland area needed to maintain bumblebee populations, by determining the quantity of ericaceous pollen required for colony development, might provide an important topic for future research.

Our results also indicate the importance of conservation of the landscape mosaic at the bumblebee foraging scale. All bumblebee species harvest pollen on species growing outside heathlands, including *Rubus*, *Sambucus* and *Tri folium*. Nevertheless, we detected no relationship between the abundance of a particular plant species in the bumblebee diet and the nutritional content of its pollen. These results show that other factors might determine foraging behaviour, although pinpointing these factors will require additional research. Quantification of floral resources at the landscape level is needed to establish bumblebee nutritional requirements and choices and to understand their foraging behaviour.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: doi: 10.1111/icad.12201.

Appendix S1. Polypeptide, amino acid, essential amino acid and sterol contents (mean ± SD; mg/g) of pollen of different plant species found in bumblebee loads in the studied heathland sites. Plant species are ranked in the order of flowering.

Appendix S2. Essential amino acid composition of pollen of 14 plant species found in bumblebee loads in the studied heathland sites, expressed as percentage of total of essential amino acids (mean ± SD). Arg = arginine, His = histidine, Ile = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Thr = threonine, Val = valine. Plant species are ranked in the order of flowering.

Appendix S3. Sterol composition of pollen of 14 plant species found in bumblebee loads in the studied heathland sites, expressed as percentage of total sterol content. 24-methyl + camp = 24-methylcholesterol and campesterol. Plant species are ranked in the order of flowering.

References


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